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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 31/65 // (A61K 31/65, 31:455)

A1

(11) International Publication Number: **WO 98/50045**

(43) International Publication Date: 12 November 1998 (12.11.98)

(21) International Application Number: PCT/GB97/01222

(22) International Filing Date: 6 May 1997 (06.05.97)

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(81) Designated States: AU, CA, HU, IL, KE, NO, NZ, PL, RO, SK, TR, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: IMPROVEMENTS IN OR RELATING TO LONG–ACTING ANTIMICROBIALS

(57) Abstract

A method for producing an improved veterinary product comprising bringing a selected long–acting antimicrobial formulation into intimate admixture with a predetermined amount of an anti–inflammatory agent and preparing the admixture for parenteral administration.

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Impr vem nts in or Relating to L ng-Acting Antimicrobials

This invention relates to administration of antimicrobials primarily in the field of veterinary medicine. The desirability of minimising administration activities in veterinary medicine is particularly acute for several reasons. Obviously the subject of treatment in veterinary medicine cannot be counselled nor cooperate in the treatment process. Therefore in addition to therapeutic considerations there arise disadvantages including the labour involved in catching and handling the animal and the stress it suffers arising from the treatment. One way of addressing these difficulties is to provide long-acting formulations so that each separate act of administration has a longer effect before a further treatment is called for.

Long acting or single treatment antimicrobials have been available for some time now in veterinary medicine. The long acting basis of such products can result from a combination of both the inherent nature of the drug or drug form used and the formulation in which it is administered. Their benefits over conventional repeat treatment products may include: reduced stress to the sick animal, in that it does not have to be caught and restrained on a daily basis in order to receive treatment, reduced work load for the farmer/veterinarian again because there are no repeat treatments and increased efficacy in treating clinical conditions in that drug levels are continuously present over a prolonged period.

However one of the problems with long acting formulations of antimicrobials is that they may cause irritation at the site of administration and also that high levels of antimicrobial can be found at the sites of administration for a long time.

A further difficulty with long acting or single treatment products can be that they may not give as high blood levels immediately following administration as do the repeat treatment products. Whilst this may not affect the overall level of efficacy of the product it may result in a

slower initial rate of recovery which can in some cases lead to the increased rate of long term damage to affected tissues and organs. An alternative explanation for the cause of tissue/organ damage is that it results as a consequence of the animals own inflammatory response to infection. As well as causing damage to tissue this inflammatory process may also reduce the diffusion of antimicrobial to the site of infection/inflammation. One means of preventing this from happening is to administer an anti-inflammatory drug. Such a drug on its own will reduce the inflammatory response but will not reduce the incidence of bacterial infection and so it is also necessary to administer an antimicrobial. One such product containing both an anti-inflammatory drug and an antimicrobial is commercially available, namely, Finabiotic, Schering-Plough Animal Health, however, use of this product requires daily treatments in order to be effective.

Accordingly it is an object of the present invention to obviate or mitigate the aforesaid disadvantages by providing a long-acting or single treatment formulation which has both antimicrobial and anti-inflammatory effect.

According to this invention there is provided a veterinary product comprising an antimicrobial and an anti-inflammatory agent in intimate admixture wherein the antimicrobial is selected from the group consisting of long-acting antimicrobials or depot antimicrobial formulations.

The product is preferably provided as a single dosage in a pharmaceutically permissible "ready for use" container such as a multi use vial or as a disposable syringe, or in packaging (e.g. blister packaging) containing a selected number of discrete dosage formulations for a prescribed period of treatment, each formulation being contained in a multi use vial or in an ampoule or disposable dispensing device adapted for parenteral use or any other suitable physiologically acceptable carrier or vehicle.

Further according to the present invention there is provided a method of producing an improved veterinary product comprising bringing a selected amount of a long

acting antimicrobial into intimate admixture with a predetermined amount of an anti-inflammatory agent and preparing the admixture for parenteral administration. Preferably the amounts of antimicrobial and anti-inflammatory agent are calculated to provide dosage amounts for a single treatment.

Advantageously this invention provides a veterinary product which on administration produces reduced irritation at the site of administration and reduced levels of antimicrobial found at the sites of administration over a period of time.

The invention will now be further described by way of reference to the following examples:

EXAMPLE 1 Comparison of a long-acting antimicrobial formulation with a formulation comprising a long-acting antimicrobial and an anti-inflammatory drug.

A trial was conducted to compare the efficacy of a long-acting antimicrobial formulation to a formulation comprising a long-acting antimicrobial and an anti-inflammatory drug. The active constituents of the two formulations are detailed in Table 1 below:

Table 1: Active constituents of formulations used in trial.

	Active ingredients
Control article	300mg/ml oxytetracycline in a long-acting formulation
Test article	300mg/ml oxytetracycline in a long-acting formulation containing 20mg/ml flunixin

The comparative efficacy of the formulations was tested using a controlled disease model of pneumonia in cattle. The object of the study was to evaluate whether the addition of anti-inflammatory drug imparted benefit to the test animals.

Sixteen cattle were inoculated over two consecutive days with cultures containing an isolate of Pasteurella haemolytica, serotype A1 from a field case of bovine respiratory disease. This organism is one of the most common bacteria associated with respiratory disease in cattle. At 48 hours after initial inoculation, fourteen animals satisfied the requirements for selection for treatment (pyrexia >103.0°F and obvious signs of respiratory disease). These animals were randomly allocated to two groups, seven animals per group. One group received a single administration of the test article (antimicrobial and anti-inflammatory), the other group receiving treatment with the control article (antimicrobial alone). Both products were administered at the same dose rate of 1ml per 10kg bodyweight on a single occasion. The study animals were maintained in their original pre-treatment pens and hence the animals from both groups were commingled in the same accommodation. The animals were closely monitored over the following days for response to treatment. This included detailed clinical examination at set timepoints by a veterinary surgeon blinded to the allocation to treatment groups. Body temperature, respiratory rate and the presence of clinical signs such as hyperpnoea (increased respiratory effort), dullness and respiratory sounds on auscultation using a stethoscope were recorded. An increase in body temperature and respiratory rate would indicate a developing respiratory disease, as would the recording of dullness, hyperpnoea and respiratory sounds. Using a pre-determined semi-quantitative weighted scoring system, the values for these five parameters were combined to give an overall score for each animal at each timepoint. The values were then summated to give a mean value per group per timepoint. The mean body temperature and total clinical score per group at timepoints considered to be representative of both short term and long term clinical efficacy are presented in Tables 2 and 3 below. The data was analysed using the Student t-t st (paired and unpaired).

Table 2: Body temperature (°F)

Timepoint	Test Article (antimicrobials + anti-inflammatory)	Control Article (antimicrobials alone)
Pre-inoculation	102.2	102.1
Pre-treatment	104.2	104.1
Post treatment		
3 hours	101.9	103.8
6 hours	101.8	103.2
9 hours	102.1	103.7
12 hours	102.3	103.9
72 hours	102.4	103.1
96 hours	102.5	103.5
144 hours	102.1	103.1

At the 3, 6, 9 and 96 hour post treatment timepoints, the values for the antimicrobial alone group were statistically significantly higher than the pre-inoculation value (paired t-test). At **none** of the above post treatment timepoints were the values for the combination product statistically significantly higher than the pre-inoculation values, indeed, at the 6 hour timepoint the values were significantly **lower** than the baseline values. Also, at the 3, 6, 9, 12 and 96 hour timepoints, the values for the test article group were statistically significantly lower than the values for the control article (unpaired t-test).

Table 3: Total Clinical Score

Timepoint	Test Article (antimicrobial + anti-inflammatory)	Control Article (antimicrobial alone)
Pre-inoculation	0.6	0.86
Pre-treatment	23.3	22.7
Post treatment		
3 hours	14.4	22.7
6 hours	13.3	19.4
9 hours	15.4	19.4
12 hours	16.7	21.6
72 hours	7.1	13.9
96 hours	4.9	14.9
144 hours	4.3	10.9

At only the last of the above post treatment timepoints (144 hours) were the values for the test group (antimicrobial alone) significantly lower than those immediately prior to the onset of treatment (paired t-test). However, at all of the above post treatment timepoints, with the exception of the 12 hour timepoint, the values for the combination product were statistically significantly lower than the values immediately prior to treatment. Also, at the 3, 6, 96 and 144 hour timepoints, the values for the test article group were statistically significantly lower than the values for the control article (unpaired t-test), the values at 72 hours being just outside statistically significance (P=0.06).

From this data, generated in a controlled acute disease model, it is clearly apparent that the combination of the long acting antimicrobial and anti-inflammatory drug had a significantly greater short term and long term therapeutic efficacy than the long acting antimicrobial alone.

EXAMPLE 2 Determination of the levels of antimicrobial in tissue.

A further trial was conducted using the formulations detailed in **Table 1** to determine the levels of antimicrobial in tissue. One half of the animals received treatment with the control article at a dose rate of 1 ml per 10 kg bodyweight and the other half received the test article at the same dose rate i.e., 1 ml per 10 kg bodyweight. At 21 and 28 days following treatment, the muscle from the injection sites (of animals from both treatments) which received a dose volume in each case of 15 ml was removed for determination of the level of antimicrobial. The results are presented below as follows:

Table 4

	Oxytetracycline concentration (μg/g) at site of administration	
	21 days	28 days
Control article	4.33	0.67
Test article	0.038	<0.025

As can be clearly seen from the results the levels of antimicrobials were considerably lower in the group treated with the test article.

EXAMPLE 3 Determination of the degree of irritation following injection.

In a further study animals were again treated with the test and control articles of **Table 1** at a dose rate of 1 ml per 10 kg bodyweight, in this case to determine the degree of irritation following injection. As well as receiving the control article, flunixin was administered as a separate formulation to the control group at a dose rate of 2 mg/kg bodyweight (the same dose as administered in the test

article). Tissue irritation was assessed by means of the determination of the levels of the enzyme aspartate aminotransferase (AST). This enzyme is released when body tissues are damaged and so its level in plasma increases. The results of the trial are presented below as follows:

Table 5

	AST Levels (u/l) After Administration:				
	0 hours	6 hours	24 hours	48 hours	96 hours
Control article flunixin at 2 mg/kg	46	81	100	83	51
Test article	39	54	67	54	44

Clearly, it can be seen in **Example 3** that the formulation of the present invention (the formulation containing the long acting antimicrobial in admixture with the anti-inflammatory drug) produced less irritation and/or tissue damage than did the long acting antimicrobial and anti-inflammatory when injected as separate products.

In the present invention the combination into one formulation of a long-acting or single treatment antimicrobial with an anti-inflammatory drug is completely novel and provides a single product which will be highly effective in the treatment of bacterial infections and associated anti-inflammatory reactions. The combination offers advantages by way of a more rapid and complete treatment of infection than delivery of either of the two individual components separately.

It should be noted that there are various possible combinations of a long-acting or single treatment antimicrobial with anti-inflammatory agent for use in veterinary medicine. In this invention the antimicrobial drug could usefully be a tetracycline, eg, oxytetracycline, a cephalosporin, or a penicillin, eg, ampicillin,

amoxycillin, penicillin G or the like or a macrolide, eg, erythromycin, tylosin, tilmicosin or the like or an aminoglycoside, eg, dihydrostreptomycin or the like, or a sulphonamide or a diaminopyrimidine eg, trimethoprim, alone or in combination. Other antimicrobials may also be usefully employed where they can exist in long-acting or single treatment formulations. The anti-inflammatory drug could usefully be flunixin, nimesulide, phenylbutazone, ketoprofen, piroxicam, dexamethasone, flumethasone, betamethasone or other drug possessing anti-inflammatory capabilities.

It will be understood by those in this art that the invention is not restricted to the particular embodiment described above, and variants based on alternative antimicrobials/anti-inflammatory agents are within the scope of the invention which is to be defined by the claims appended hereto.

CLAIMS

1. A method for producing an improved veterinary product comprising bringing a selected long-acting antimicrobial formulation into intimate admixture with a predetermined amount of an anti-inflammatory agent and preparing the admixture for parenteral administration.

2. A method according to claim 1 wherein the amounts of antimicrobial and anti-inflammatory agent are calculated to provide an appropriate dosage for a single treatment.

3. A veterinary product produced by the process of claims 1 or 2 comprising an antimicrobial and anti-inflammatory agent, in intimate admixture.

4. A veterinary product according to claim 3 wherein the antimicrobial is selected from the group consisting of tetracyclines such as oxytetracycline, cephalosporins, penicillins such as ampicillin, amoxycillin, penicillin G or the like, macrolides such as erythromycin, tylosin, tilmicosin or the like or aminoglycosides such as dihydrostreptomycin or the like or a quinolone or the like, or a sulphonamide or a diaminopyrimidine eg, trimethoprim or the like, either alone or in combination.

5. A veterinary product according to claim 4 wherein the anti-inflammatory agent is selected from the group consisting of indolines such as indomethacin, salicylates such as aspirin, oxicams such as piroxicam, acetic acid such as diclofenac, fenamates such as tolfenamic acid, propionic acids such as ketoprofen, para amino phenol derivatives such as acctaminophen, pyrazoles such as phenylbutazone, sulphonanilides such as nimesulide, or other drugs possessing anti-inflammatory or anti-pyretic capabilities.

6. A veterinary product comprising a long-acting antimicrobial, flunixin and a physiologically acceptable vehicle or carrier.

7. A veterinary product according to claim 6 comprising oxytetracycline, flunixin and a physiologically acceptable vehicle or carrier.

8. A veterinary product according to claim 7 comprising 300mg/ml oxytetracycline, 20mg/ml flunixin and a physiologically acceptable vehicle or carrier.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/01222

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/65 //(A61K31/65,31:455)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LOCKWOOD P W ET AL: "Flunixin meglumine as an adjunct to antibacterial therapy: Efficacy in the treatment of the bovine respiratory disease complex under typical North American feedlot conditions" TIERAERZTLICHE UMSCHAU, 52 (3). MAR 1997. 127-128, 130-131., XP002052672 see abstract ---	1-8
A	DOHERTY M.L. ET AL: "Isolation of Mycoplasma bovis from a calf imported into the Republic of Ireland" VET. REC., 1994, 135/11 (259-260), UNITED KINGDOM, XP002052673 see page 259, column 1, paragraph 3 --- -/--	1-8

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search
20 January 1998

Date of mailing of the international search report
27.02.98

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 97/01222

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VERHOEFF J. ET AL: "Flunixin meglumine in calves with natural bovine respiratory syncytial virus infection" VET. REC., 1986, 118/1 (14-16), ENGLAND, XP002052674 see page 15, column 2, paragraph 2 ---	1-8
A	KOPCHA M. ET AL: "Experimental uses of flunixin meglumine and phenylbutazone in food-producing animals" J. AM. VET. MED. ASSOC., 1989, 194/1 (45-49), USA, XP002052675 see page 47, column 1, paragraph 2 ---	1-8
A	WO 96 01634 A (NORBROOK LAB LTD ;PATTERSON ALAN (GB); HOLMES DREW (GB)) 25 January 1996 see abstract -----	1-8

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 97/01222

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-6
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

In view of the very large number of compounds which are defined by the wording of the claims, the search has been performed on the general idea and the combination explicitly mentionned in claim 7 and 8

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter nal Application No
PCT/GB 97/01222

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9601634 A	25-01-96	AU 2804395 A	09-02-96
		CA 2194576 A	25-01-96
		EP 0769951 A	02-05-97